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RESEARCH ARTICLE

**ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS AGAINST MULTI DRUG RESISTANT
URINARY TRACT INFECTION CAUSING ORGANISMS**

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Abstract

The present study was isolate Bacterial pathogens form Urinary Tract Infection and identified the Bacterial pathogens from UTI patients. Determination of the antibiotic drug resistant pattern of the isolated pathogenic bacteria using standard antibiotic discs Ampicilin (25µg), Erithromycin (15µg), Chloramphenicol (10µg) Gentamicin (10µg) and Tetracycline (30 µg).The study was carried out, in vitro screening of ethanolic extracts of some medicinal plants against the bacterial pathogens Escherichia coli, Proteus vulgaris, Staphylococcus aureus and Pseudomonas aeruginosawere isolate from the UTI. When compared with standard antibiotic disc selected plants extracts were showed maximum zone of inhibition against all the pathogens. This investigation strongly recommends that phytochemical studies are required to determine the types of compounds responsible for the antibacterial effect of these medicinal plants.

Key words: Bacterial pathogens, Antibiotic drug resistant pattern and Medicinal plants

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Introduction

Irresistible infections are a significant wellbeing risk from one side of the planet to the other, both in creating and created nations. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. A number of researches now a day are working seriously to find out substitutes for antibiotics as they cause side effects on the functioning of different parts of the body, organs and systems.

Urinary Tract Infection (UTI) alludes to both microbial colonization of the pee and tissue intrusion of any construction of the urinary parcel. Bacteria are most commonly responsible although yeast and viruses may also be involved. Urinary Tract Infections (UTIs) are second most common type of infection in the body, a leading cause of grimness and medical services uses in people, everything being equal. Physically dynamic young ladies are excessively influenced, yet a few different populaces, including older people and those going through genitourinary instrumentation or catheterization, are additionally in danger.

Urinary parcel diseases are somewhat normal issues during pregnancy.. The physiologic changes related to pregnancy make otherwise healthy women susceptible to serious infectious complications, arising from conditions such as asymptomatic and symptomatic urinary tract infections.

Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging from asymptomatic presence of bacteria in the urine to severe of the kidney with sepsis. UTIs are one of the most common bacterial infections in humans both in the community and hospital settings (Dalela, *et al.*, 2012). Around the world, roughly 150 million individuals are determined to have UTIs bringing about USD 6 billion medical services uses. UTIs are the most common bacterial infections encountered by clinicians in developing countries.

Medication obstruction in microorganisms is a predictable and maybe inevitable reaction to the utilization of antimicrobial specialist. It can arise from the selection of resistant strains among naturally susceptible species or from the ingress of new strains of naturally resistant species. The extent of use of particular agents in a given environment dictates the rate at which resistance arises among microbial populations (Kuninet *al.*, 1990). Some organisms rapidly acquire resistance e.g. coliforms and *Staphylococcus aureus*, while others rarely do so e.g. *Streptococcus pyogenes* (Sleigh and Timbury, 1986). The emergence of drug resistant bacteria is a major problem in antibiotic therapy.

Plant based antimicrobials address an immense undiscovered hotspot for medications.

Proceeded and further investigation of plant antimicrobials needs to happen. Plants based antimicrobials have enormous therapeutic potential and they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. They are effective, yet gentle. Many plants have tropisms to specific organs or system in the body. Phytomedicines typically effectsly affect the body. Their actions often act beyond the symptomatic treatment of disease. An example of this is *Hydrastiscanadensis*. *Hydrastis* has antimicrobial movement, yet additionally builds blood supply to the spleen advancing ideal action of the spleen to deliver interceding compounds (Murray, 1995).

As indicated by World Health Organization (WHO) over 80% of the total populace depends on conventional medication for their essential medical services needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Zaika, 1975).

In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments India officially recognizes over 3000 plants for their medicinal value. Today, there is far and wide interest in drugs got from plants. This interest principally comes from the conviction that green medication is protected and reliable, contrasted and exorbitant manufactured medications that have unfavorable impacts (Gordan and David, 2001).

In developing countries low income people depend on the use of plants in traditional healing by either tribal people or indigenous communities of Tamil Nadu (Natarajan *et al.*, 1999 and Ignachimuthu *et al.*, 1998) Traditional healers claim that their medicine is cheaper and more effective than modern medicine. In the emergence of drug resistance in human pathogenic organisms, there is a need to foster elective antimicrobial medications for the treatment of irresistible infections. One methodology is to screen new, reasonable and viable medications from different sources, including plants, for conceivable antimicrobial properties. Keeping this in mind the present study is carried out to evaluate

antibacterial activity of some medicinal plants against drug resistant Urinary Tract Infection causing organisms. This study was designed to determine the prevalence and antibiotic susceptibility patterns of common urinary bacterial isolates.

Materials and method

The present study pertains to the isolation of pathogens from Urinary Tract Infection (UTI). Urine samples were collected from patients of all age groups (Table – 1) referring in Government hospital at Tiruvannamalai, Tamil Nadu, India.

Collection of Urine Specimens

Early morning clean catch Mid stream urine was collected in a sterile, dry leak proof container and transported to the laboratory as soon as possible. If suspected delay the sample was stored at 4°C.

Transport of Specimen

After collection Specimens were transported to the laboratory with the minimum of delay preferably within 2-3 hours of collection. In the laboratory specimens were preserved in the refrigerator at 4°C before processing.

Sterilization of Glassware and Media

In all experiments standard microbiological techniques were followed for the sterilization of glassware and media.

Isolation of Bacterial pathogen

The urine sample was inoculated in Nutrient agar medium, Blood agar medium and MacConkey medium. The plates were incubated at 37°C for 24 hrs and the colonies were observed. Morphologically distinct colonies were picked up and stored in nutrient agar slants for further investigation.

Identification of Bacterial isolate

Identification of the bacterial pathogen was done by the following routine cultural practices and biochemical tests (Aneja, 1996).

Micro Organisms

For the present study pertaining to isolation of *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* from the UTI (Urinary Tract Infection) patient urine was collected.

Antibacterial sensitivity test

Inoculum preparation

Stock cultures were maintained at 4°C on slopes of Nutrient agar. Active cultures for experiments were prepared by transferring a loopfull of cells from the stock cultures to sterile Nutrient broth and incubated at 37°C for 6hrs. After incubation the concentration of the broth was adjusted to McFarland scale 0.5 with 0.85% NaCl solution and confirmed by spectrophotometric reading at 580 nm. Cell suspensions were finally diluted to 10^4 UFC.ml⁻¹ for being used in the activity assays.

Antibiotic activity of standard antibiotics

Sterile Nutrient Agar plates were prepared. The culture was taken with the help of sterile cotton swab and swabbed aseptically on plates three times by rotating the plates at 60° angles. The inoculum was allowed to dry for 1 minute and the antibiotic discs Ampicillin (25µg), Erythromycin (15µg), Chloramphenicol (10µg) Gentamicin (10µg) and Tetracycline (30 µg) were placed on the surface of the inoculum. After incubation the plates were observed for inhibition zone and the diameter of the zone was measured in millimeter the results were recorded and tabulated.

Preparation of Plant Extracts

The methodology was followed as per Onkarappa *et al.*, (2003) with slight modifications. The collected plant materials (Table – Medicinal plants) are thoroughly washed and air dried. 50% ethanol was used for extract preparation, 5 gm of fresh plant material was extracted in 20 ml of ethanol. The extract was filtered through Whatmann filter paper No.1, then the filtrate was centrifuged at 5000 rpm for 5 minutes. The supernatant was collected in sterile flasks and was stored in refrigerator.

Table – Medicinal Use of Selected Medicinal Plants

S. No.	Name of the Plant	Family	Habit	Vernacular Name	Parts used	Medicinal Uses
1	<i>Asparagus racemosus</i>	Asparagaceae	Climber	Thanier vittan kilangu	Tuberous root	Leprosy, Epilepsy, Cough, Bronchitis, Gonorrhea, Scalding of urine, Hepatopathy, Throat infection, Diarrhea, Dysentery, Tumors and Nervous disorders.
2	<i>Catharanthus roseus</i>	Apocynaceae	Shrub	Nithya kalyani	Whole Plant	Diabetes, Stomachache, wasp Stings and Leukemia.
3	<i>Cissus quadrangularis</i>	Vutaceae	Climber	Pirantai	Whole Plant	Chronic ulcers, Leprosy, Skin diseases, Scurvy, asthma, burns and wounds, tumors hemorrhages, epilepsy and swellings.
4	<i>Croton bonplandianum</i>	Euphorbiaceae	Herb	Rail pundu	Leaves	Skin diseases, cuts and wounds.
5	<i>Datura metel</i>	Solanaceae	Shrub	Karuummattai	Whole Plant	Asthma, Fever, Ulcer, Skin diseases, Mumps, painful swellings, epilepsy, cephalgia, dandruff and to treat bites from rabid dogs.
6	<i>Delonix elata</i>	Caesalpinioideae	Tree	Vadanarayanan	Leaves	Rheumatism and Flatulence
7	<i>Euphorbia hirta</i>	Euphorbiaceae	Herb	Amampatcharirisi	Whole Plant	Asthma, cough, dysentery and diseases of Genitourinary tract
8	<i>Ficus racemosa</i>	Moraceae	Tree	Atti	Roots, Leaves, Fruits	Dysentery, wounds, ulcer, diabetes, diarrhea, abortions and hemorrhages.
9	<i>Solanum nigrum</i>	Solanaceae	Herb	Manattakkali	Whole Plant	Fever, swelling, asthma, ulcers, wounds, leprosy, skin disease, vomiting, bronchitis, cough and splenomegaly.
10	<i>Thespesia populnea</i>	Caesalpinioideae	Tree	Puvarasam	Whole plant	Skin diseases, wounds, ulcers, diarrhea, dysentery, cholera, diabetes, cough, asthma and hemorrhages.
11	<i>Withania somnifera</i>	Solanaceae	Shrub	Amukkara	Roots, Leaves	Constipation, fever, painful swelling, ulcers and carbuncles.

In vitro screening for antibacterial activity

Culture plates for bacteria are prepared by pouring 15-20 ml of Nutrient agar medium into sterile petriplates. The test bacteria were inoculated into nutrient broth and incubated at 37°C for 24 hours. After the incubation period the culture tubes were compared with the Turbidity (opacity) standard.

In vitro antibacterial activity of plant extracts was tested by disc diffusion methods. The inoculum suspension was spread uniformly over the agar medium using sterile cotton swabs to get uniform lawn of bacteria. Then the plates were incubated for 24 hours at 37°C. The effectivity of these extracts was recorded by measuring the diameter of inhibition zone at the end of 24 - 48 hours incubation.

Result

The most frequent agents of severe bacterial infections (UTI) were isolated by the standard procedures and the identification was done on the basis of the scheme outlined by Bergey's Manual of Systematic Bacteriology, cultural characters and biochemical characters of the isolates on the selective media. *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolate from the UTI. The results are presented in Table – 1, 2 & 3.

Table 1: Samples for Urinary Tract Infection of Women

Age-group	Women
16-20	3(12%)
21-30	2(8%)
31-40	4(16%)
41-50	1(4%)

The samples of urine were collected from infected women. Age and number of sample was 16 – 20 age group 2 (8%), 21 – 30 age group 3 (12%), 31 – 40 age group 4 (18%) and 41 – 50 age group 1 (4%) observed in selected samples.

The antibiogram of the clinical isolates was done with standard antibiotic discs Ampicillin (Amp) 25 µg, Chloramphenicol (Chl) 10 µg, Erithromycin (E)

15 µg, Gentamicin (G) 10 µg, Tetracycline (T) 30 µg. (Table – 4)

The antibiogram study of the bacterial isolates to various antibiotics clearly reveals that the isolates are exhibiting resistance to various antibiotics against *Escherichia coli* and *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Table - 4).

Antibiotic sensitivity test pattern of four bacterial pathogens isolated from UTI is as per test result showed in table – 4. *Escherichia coli* was resistant to three antibiotics tested Ampicillin (-), Erithromycin (18mm), Tetracycline (14mm). But intermediate against Chloramphenicol (18mm), Gentamicin (19mm). *Proteus vulgaris* was resistant to all the tested antibiotics Ampicillin (8mm), Chloramphenicol (18mm), Erithromycin (-), Gentamicin (6mm), Tetracycline (10mm).

Pseudomonas aeruginosa were showed resistant to the tested antibiotics Ampicillin (5mm), Chloramphenicol (15mm), Erithromycin (18mm), Gentamicin (6mm), Tetracycline (10mm). *Staphylococcus aureus* were resistant to all the tested antibiotics Ampicillin (17mm), Chloramphenicol (20mm), Erithromycin (15mm), Gentamicin (18mm), Tetracycline (20mm) table - 4.

The isolated bacteria showed a very high rate of resistance to standard antibiotic discs Ampicillin, Chloramphenicol, Erithromycin, Gentamicin, Tetracycline. So the antibiotic activity of medicinal plant extract was done by disc diffusing test.

Antibacterial Activity of Plant Extracts

The results of the antibacterial screening of selected medicinal plant species were given in Table – 3.

Ethanol extract of *Asparagus racemosus* showed maximum inhibition zone in *Pseudomonas aeruginosa* (21 mm) followed by *Proteus vulgaris* (17 mm) and *Escherichia coli* (14 mm) respectively. No Inhibition zone was found in *Bacillus subtilis*.

Catharanthus roseus leaves exhibited minimum inhibitory zone in *B. subtilis* (12 mm) and *E. coli* (16 mm), whereas flower extracts expressed activity against *B. subtilis* (18 mm) and *E. coli* (8 mm). No

antibacterial activity was recorded against other organisms.

There is moderate activity of *Cissusquadrangularis* against all the bacterial Pathogens except *P. vulgaris*. While in *Croton bonplandianum* and *Solanumnigrum* extracts were active against *Pseudomonas aeruginosa* (20, 14, 18 mm and 15, 15, 18mm). All the other pathogens were resistant to the extracts.

Extracts from aerial parts of *Daturametel* (Leaves, Flower, Fruit and Stem) exhibits antibacterial activity against the bacterial pathogens namely, *E. coli* (12 mm, 10 mm, 14 mm and 16 mm), *P. vulgaris* (8 mm, 14 mm and 8 mm), *B. subtilis* (6 mm, 10 mm, 20 mm and 8 mm) and *P. aeruginosa* (18mm, 13 mm and 22 mm) respectively. Among these extracts, leaf extract fails to inhibit the growth of *B. subtilis*, followed by flower and stem extracts against *P. aeruginosa* and there is no activity in fruit extracts, against *B. subtilis*, *P. aeruginosa* and *P. vulgaris*.

Leaf extracts of *Delonixelata* and *Euphorbia hirta* showed same results with slight variations. But in case of *Delonixelata* inhibited the growth of *B. subtilis*, *E. coli* and *P. aeruginosa* (6 mm, 10 mm, 15 mm and 16 mm) whereas *E. hitra* (9 mm, 10 mm and 20 mm) respectively. Both the extracts had no response against *P. vulgaris*.

The fruit extract of *Ficusracemosa* showed activity against *B. subtilis* and *P. aeruginosa*(18 mm, 15 mm and 19mm) whereas it does not respond to other two pathogens.

The ethanolic leaf extracts from *Solanumsurattense* and *Tabernaemontanadivaricata* were observed that similar inhibitory activity against *P. aeruginosa* (15 mm and 14 mm) whereas *S. surattense* was active against *E. coli* (12 mm) and *T. divaricata* against *B. subtilis* (9 mm). But the flower extracts showed minimum inhibition zone against *B. subtilis* (9 mm) and no activity was recorded with other organisms.

Thespesiapopulnea flower bud extracts exhibit good antibacterial activity against all the four test organisms namely *B. subtilis*(17 mm), *E. coli* (15 mm) *P. aeruginosa* (9 mm) and *P. vulgaris* (13 mm).

While in the leaf extracts of *WithaniaSominiifera* was sensitive to *E. coli* (20 mm) and *P. aeruginosa* (18 mm). There is good inhibition zone against *B. subtilis*(15 mm) and *P. vulgaris* (15 mm).

100 clinical specimens were investigated for the presence of *Staphylococcus aureus*. The frequency of isolation of *Staphylococcus aureus* from the different specimens analyzed is given in table 1; a total of 48 isolates of *Staphylococcus aureus* were isolated. The isolates were most delicate to gentamicin (91.7%), cloxacillin (85.4%) and most resistant to penicillin (95.8%) and ampicillin (89.6%). The limit of *Staphylococcus aureus* to produce human infections has not diminished with the presentation of anti-infection agents. The life forms show astounding versatility in their conduct towards anti-infection agents (Grassi, 1988), with a few strains having defeated most commonly used drugs. In this study, a high sensitivity percentage to gentamicin (91.7%), Cloxacillin (85.4%) was recorded. Additionally the majority of the strains of *Staphylococcus aureus* were delicate to erythromycin (66.7%) and streptomycin (66.7%).

This study supports the contention that traditional medicinal plants have a valuable resource in the potential discovery of novel drugs through ethnopharmacology (Heinrich, 2000; Heinrich and Siman, 2001). Because of the development of medication safe strains of numerous irresistible microorganisms, ethnobotany may give new, viable drug options in contrast to existing medications. In the present study, urinary tract infection has been selected because these two problems have been reported to be the most common problem. Patton *et al.* (1991) has reported that UTIs are the leading cause of gram negative bacteremia.

Forty-eight strains of *Staphylococcus aureus* were isolated from clinical sources. The specimens were obtained from different patients under medical attention. The specimens obtained from urine samples. The samples were streaked on Mannitol Salt Agar (MSA) and blood agar (BA) plates. The plates were all incubated at 37°C for 24 hours, after which the cultural and morphological characteristics of the isolates were studied. Identification of isolates was by standard microbiological methods as described by Cowan (1993).

Table 2: Biochemical test for Isolated Pathogens from UTI sample

S. No.	Organisms	Ox	UR	MR	VP	Cit	Ind	Gel	Nit	TSI Agar				Glu	Lact
										Slope	Butt	H ₂ S	Gas		
1	<i>E. Coli</i>	-	-	+	-	-	+	-	+	Y	Y	-	+	+	+
2	<i>P. vulgris</i>	-	+	-	-	+	-	+	+	R	Y	+	+	+	-
3	<i>P. aeruginosa</i>	+	D	-	-	+	-	+	+	R	R	-	-	+	-
4	<i>Staphylococcus aureus</i>	-	-	+	-	-	-	-	+	R	Y	-	+	+	-

Table 3: Pathogenic bacteria Isolated from Urinary Tract Infection

S. No.	Name of pathogens	Female
1.	<i>Escherichia coli</i>	4(40%)
2.	<i>Proteus vulgaris</i>	3(30%)
3.	<i>Pseudomonas aeruginosa</i>	2(20%)
4.	<i>Staphylococcus aureus</i>	1(10%)

Table 4: Antibacterial activity of standard antibiotic disc against Isolated Organisms of UTI

S.No	Antibiotics	Zone of inhibition (Diameter in mm)			
		<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
1	Ampicillin	-	8	5	17
2	Chloramphenicol	20	18	15	20
3	Erithromycin	18	-	18	15
4	Gentamicin	19	6	20	18
5	Tetracycline	14	10	17	20

Table - 5: Antibacterial Activity of Selected Medicinal Plants against Isolated Organisms of UTI

S. No.	Name of the Plant	Parts Used	Zone of Inhibition (Diameter in mm)			
			<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
1	<i>Asparagus racemesus</i>	Leaves	14	17	21	-
2	<i>Catharanthus roseus</i>	Leaves	16	13	20	12
		Flower	8	13	17	18
3	<i>Cissus quadrangularis</i>	Stem	17	-	-	15
4	<i>Croton bonplandianum</i>	Leaves	18	4	14	20
5	<i>Datura metel</i>	Leaves	12	8	18	6
		Flower	10	14	13	10
		Fruit	14	-	22	20
		Stem	16	8	-	8
6	<i>Delonix elata</i>	Leaves	15	-	16	10
7	<i>Euphorbia hirta</i>	Leaves	9	-	20	10
8	<i>Ficus racemosa</i>	Fruits	-	19	15	18
9	<i>Solanum nigrum</i>	Fruits	-	15	15	18
10	<i>Thespesia populnea</i>	Flower bud	9	13	15	17
11	<i>Withania somnifera</i>	Leaves	20	15	18	15

Most UTIs are caused by Gram-negative bacteria like *Escherichia coli* (*E. coli*), *Klebsiella* spp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Serratia* spp. and Gram-positive bacteria such as *Enterococcus* spp. and *Staphylococcus* spp. (Theodros. 2010). *E. coli* is responsible to most UTIs (Beyene, and Tsegaye. 2011, Demile, *et al.*, 2012). Drug resistance among bacteria causing UTI has increased since introduction to UTI chemotherapy (Nerurkar, *et al.*, 2012, Sood and Gupta. 2012, Bahadin, *et al.*, 2011).

The etiological agents and their susceptibility patterns of UTI vary in regions and geographical location. Besides, the etiology and drug resistance change through time (De Francesco, *et al.*, 2007). Information on the neighborhood bacterial etiology and defenselessness designs is needed to follow any change that may have happened in time so that refreshed proposal for ideal exact treatment of UTI can be made (Leegaard, *et al.*, 2000). In Ethiopia, various investigations have been done on the commonness and antimicrobial obstruction examples of UTIs (Kashef, *et al.*, 2010, Theodros. 2010, Nerurkar, *et al.*, 2012). However, data have been reported from the study area. The aim of the study was therefore to determine the prevalence of local bacterial isolates from suspected UTI and susceptibility to the most commonly used antimicrobials.

Conclusion

The present study on the bacteriological profiles of the urinary tract infections showed that the rate of such infections is high, even though it was within the reported range. Protection from antimicrobials represents a genuine and developing issue, on the grounds that such safe microscopic organisms are turning out to be more hard to treat. The empirical and the indiscriminate use of antibiotics should be avoided in order to curtail the emergence and the spread of drug resistance among pathogens. Reduction of infections and antimicrobial resistance is both a challenge and goal of all around the world. Severe disease control estimates like all inclusive precautionary measures and tough adherence to hand washing rehearses, definition of anti-microbial arrangement,

observation exercises, may be needed for something similar.

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